

PATENT  
Customer No. 22,852  
Attorney Docket No. 07680.0031-00000

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

1-22. (Canceled)

23. (Currently amended) A method for coupling a an oligosaccharide comprising a phosphorylated ~~hexose~~ mannose to a lysosomal enzyme, the method comprising the steps of:

- (a) derivatizing the oligosaccharide ~~comprising a phosphorylated hexose with a compound containing~~ to generate a carbonyl-reactive group;
- (b) oxidizing the lysosomal enzyme to generate at least one carbonyl group on the lysosomal enzyme; and
- (c) reacting the derivatized oligosaccharide with the oxidized lysosomal enzyme,

thereby coupling the oligosaccharide to the lysosomal enzyme.

24. (Currently amended) The method according to claim 23, wherein the ~~phosphorylated hexose phosphate group is linked to~~ is a terminal mannose hexose.

25. (Currently amended) The method according to claim 23, wherein the ~~phosphorylated hexose phosphate group is linked to~~ is a penultimate mannose hexose.

26. (Canceled)

27. (Currently amended) The method according to claim 23, wherein the oligosaccharide comprises two or more M6P mannose-6-phosphate (M6P) groups.

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28. (Previously presented) The method according to claim 23, wherein the oxidizing step is carried out with periodate or galactose oxidase.

29. (Currently amended) The method according to claim 23, wherein the lysosomal enzyme is deficient in a lysosomal storage disease chosen from Fabry disease, Pompe disease, Tay-Sachs disease, Hurler disease, or Hurler-Scheie disease, Krabbe disease, Hunter disease, Mmetachromatic leukodystrophy, Sanfilippo A, and Sanfilippo B disease, Morquio disease, Maroteaux-Lamy disease, and Gaucher disease.

30. (Previously presented) The method according to claim 23, wherein the lysosomal enzyme is chosen from beta-glucocerebrosidase, alpha-galactosidase A, acid alpha-glucosidase, alpha-N-acetylglucosaminidase, beta-N-acetyl-hexosaminidase, and beta-glucuronidase.

31. (Previously presented) The method according to claim 23, wherein the oligosaccharide is chosen from a biantennary mannopyranosyl oligosaccharide and a triantennary mannopyranosyl oligosaccharide.

32. (Previously presented) The method according to claim 31, wherein the biantennary mannopyranosyl oligosaccharide comprises bis-M6P.

33. (Previously presented) The method according to claim 31, wherein the triantennary mannopyranosyl oligosaccharide comprises bis-M6P or tri-M6P.

34. (Currently amended) The method according to claim 23, wherein the oligosaccharide comprises:

~~6-P-M (alpha-1,2)-M(alpha-1,3)~~

~~M~~

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6-P-M(alpha 1, 2)-M(alpha 1, 6)

6-P-M(alpha 1, 2)-M(alpha 1, 3)

M

6-P-M(alpha 1, 2)-M(alpha 1, 6)

wherein M is mannose or a mannopyranosyl group.

35. (Currently amended) The method according to claim 23, wherein the derivatized oligosaccharide has a formula chosen from  $6\text{-P-}M_n\text{-}R$  and  $(6\text{-P-}M_x)_mL_n\text{-}R$ , wherein M is mannose or a mannopyranosyl group, P is a phosphate group linked to the C-6 position of M, L is a hexose, R is a compound containing has at least one carbonyl-reactive group, m is an integer ranging from 2 to 3, n is an integer ranging from 1 to 15, wherein if  $n > 1$ , the  $M_n L_n$  are linked to one another by alpha (1,2), alpha (1,3), alpha (1,4), or alpha (1, 6), and x is an integer ranging from 1 to 15.

36. (Previously presented) The method according to claim 35, wherein at least one L is mannose.

37. (Previously presented) The method according to claim 35, wherein at least one L is chosen from galactose, N-acetylglucosamine, and fucose.

38. (Currently amended) The method according to claim 23 or claim 35, wherein the compound containing at least one carbonyl-reactive group is chosen from a hydrazine, a hydrazide, an amineoxyl aminoxy, a semicarbazide semicarbazide.

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39. (Previously presented) The method according to claim 23, further comprising the step of adding a reducing agent to the coupled lysosomal enzyme.

40. (Previously presented) The method according to claim 39, wherein the reducing agent comprises cyanoborohydride.

41. (New) The method of claim 23, wherein the lysosomal enzyme is deficient in Pompe disease and the carbonyl-reactive group is chosen from a hydrazine, a hydrazide, an aminoxy, a semicarbazide.

42. (New) The method of claim 23, wherein the lysosomal enzyme is acid alpha-glucosidase.

43. (New) The method of claim 23, wherein the carbonyl-reactive group is aminoxy.